

EFFECT OF COLCHICINE ON THE DEVELOPMENT OF THE FISH EMBRYO, *ORYZIAS LATIPES*

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I

The alkaloid, colchicine, has recently come into prominence biologically because of its effects on mitosis. In plants it produces polyploidy, while in animals it not only checks mitosis (Allen et al., 1937) but also appears to stimulate the mitotic rate (Paff, 1939; Havas, 1939; Mills, 1939). When introduced into the hen's egg of 48-hour incubation (Paff), the resultant effects include distortion due to overgrowth and abnormalities of the vascular system and body flexures. In the frog embryo, colchicine provokes a meroblastic type of cleavage, disturbs or limits gastrulation and produces enlarged cells (Keppel and Dawson, 1939). The embryos did not develop beyond the neural fold stage. Bastenie and Zylberszac (1938) have shown that it stimulates the parathyroid gland, while in male fish, Havas (1939) has found that colchicine provokes color change of the same intensity as testis hormone.

This paper is a report of colchicine effects upon the development of the fish embryo, *Oryzias latipes*. Attention was focussed particularly upon the differentiation of embryonic structures. All cultures were followed until the embryos either hatched or died. Exposure in the dark at room temperature was begun both before and after the onset of differentiation to different concentrations and for varying periods of time, after which the embryos were transferred to fresh water. In some cases exposure was continuous, while in others the chorion was pricked. During a period of continuous exposure in the dark, the colchicine solution was changed every 2-3 days.

II

Table I includes an outline of the experiments with cleavage stages together with a brief summary of the results based upon the use of toxic concentrations of the alkaloid. Non-toxic concentrations showed no stimulatory action on the development of this fish. In a 1-100,000 concentration development from a cleavage stage was only slightly retarded

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during a continuous exposure and the embryos hatched about 3 to 5 days later than the control. Increasingly stronger concentrations caused progressively greater retardation and the weakened embryos failed to hatch.

TABLE I
Effect of colchicine on early development of Oryzias latipes.
(Cleavage stages)

Develop- mental stage	Conc.	Length of ex- posure	No. eggs used	No. living at end of ex- posure	No. living at end of ex- per- iment	Time in fresh water	Results
Early cleavage	1-100,000	25 days	23		18		7 hatched by 25 days, weak and died within a few hours.
	1-10,000	10 days	14	none		3 days	Young optic cup stage. Trunk region reduced and tail absent. Dead.
	1-5,000	9-48 hrs.	82	prob- ably 34		16 hrs.	Little differentiation. Increase in cell number and some axial thickening. Few in neural tube or optic vesicle stages. Died.
Medium cleavage	1-100,000	12-25 days	47	30			Slight retardation only. 7 hatched by 25 days.
	1-10,000	13 days	52	7		10 days	Small size. Reduced or spindly trunk and tail. Somites obscure. Trunk queerly bent. Yolk vascularization reduced or absent. One had weakly beating heart.
	1-5,000	2 hrs.	26	all	24	25 days	No retardation evident. 7 hatched by 22 days
Medium cleavage		4 hrs.	35		35	30 days	Slight retardation. 8 hatched by 27 days.
	1-5,000	20 hrs.				55 hrs.	All dead. Some had developed to optic vesicle stage with reduced tails.
		27 hrs.	93	43	none	19-33 hrs.	Many failed to recover. Few developed to non-pigmented eye stage. Trunk of varying length. All dead by 10 days.
Late cleavage		44 hrs.				31 hrs.	All dead. No recognizable differentiation.
	1-5,000	22.5 hrs.	67	60	35	21 hrs.	Only few more cleavages during exposure. After transfer = 7 in embryonic shield stage, 6 undifferentiated, 4 in short neural tube stage. Living remainder only slightly retarded.
		7 days	34	none			Optic vesicle to young optic cup stage. Reduced trunk and no tail development. Died.
	1-1,000	1 hr.	15	all?	4	8 days	Typical appearance in 4. Others died.
		2 hrs.	21	all?	6	8 days	3 retarded but typical. 4 with reduced trunk and tail, cycloplan-like eye, abnormal brain.
		3.5 hrs.	29	all?	none	2 days	No development in majority. 6 are in short neural tube stage.
			18	all?	6	7 days	5 retarded, 2 cycloplan cases, 5 lack tail with short trunk, small eyes with little pigment.
		9.5 hrs.	23	all?	none	7 days	No differentiation and no recovery.
		19 hrs.	52	all?	1	1 day	Further cleavage but no differentiation. Those left in colchicine are dead.

One to 10,000 caused great general retardation of size, differentiation, developmental rate, pigmentation of the eye and body, etc. It also inhibited and retarded the growth of the trunk region and tail, the formation of somites and extra-embryonic vascular vessels. The latter effect has been reported for the chick embryo by Paff (*loc. cit.*) who began exposure at the 48-hour stage. In many fish the rudimentary tail was strangely bent or twisted. Apparently there occurred a greater interference with the development of the trunk and especially the tail than with the head region. Following an exposure to 1–5,000 these effects were much accentuated while the tail might be entirely lacking. This solution acted very quickly since during exposures of 23 to 48 hours only a few cleavages took place while the eggs were in solution. The yolk became more or less covered by a cellular layer but no differentiation occurred. Embryos may adapt themselves somewhat to continuous exposure at this concentration and stage, but, as will appear later, older embryos showed greater capacity in this respect and even in a 1–1,000 concentration differentiation continued.

Exposure of cleavage stages for as short a period as 2 hours to a concentration of 1–1,000 subsequently produced small embryos with reduced trunk and tail, cyclopiian-like eyes, reduced and uneven distribution of pigment in eyes and body, and abnormal brain which was frequently bent or folded. During the exposure period further development was limited to a few cleavages. About 4 hours later the majority failed to recover, while in fresh water a few embryos went as far as a shortened neural tube stage. The effect on the eyes was especially striking. The pigmentation was much reduced or lacking, while in the cyclopiian-like eyes the pigment appeared as small, densely aggregated, irregularly-shaped masses at the anterior region. These masses varied from a large single one to two variously sized smaller ones connected by a line of pigment. A few such cases were secured from late cleavage stages but not from stages in which differentiation had begun.

The production of cyclopiian eyes in animals has been discussed by Adelman (1936) and apparently a wide variety of chemical substances are causative agents. Stockard (see Adelman) concluded from his experiments on *Fundulus* that the action of chemicals must be exerted relatively early since eggs treated later than 15 hours after fertilization failed to respond, the most effective period being the 8–32 cell stage. The present results agree with this conclusion as more cases were secured from the earliest cleavage stages. Certain of the types of developmental modification produced in the fish embryo are comparable to those caused by x-rays and 2,4-dinitrophenol (Waterman, 1939).

The effect of colchicine is related to the concentration, length of exposure, and the stage of development. Three other developmental stages

have been tested for comparison with the cleavage stages, i.e. the neural fold or tube, the optic vesicle and the optic cup stage previous to the appearance of pigment. Reference to Table II will show that these older stages have progressively greater survival capacity for comparable con-

TABLE II
Effect of colchicine on early development of Oryzias latipes.
(Neural fold to optic cup stages)

Developmental stage	Conc.	Length of exposure	No. eggs used	No. living at end of exposure	No. living at end of experiment	Time in fresh water	Results
Neural fold	1-5,000	23 hrs.	43	all		20 days	23 hatched. Little if any retardation.
	1-1,000	11 hrs.	57	all?	9	5 days	Reduced or abnormal trunk and tail, no extra embryonic vascular vessels. Tail often lacking.
		21 hrs.	48	all?	none	2 days	In solution = retarded, reduced tail and small trunk, optic cup stage lacking pigment. Died.
		23.5 hrs.	41	all?	4	5 days	Development in sol. In fresh water one was typical, but small. 3 had reduced tails. Died.
Neural tube	1-1,000	13 hrs.	52		21	76 hrs.	Cycloplan-like distribution of eye pigment. Large ear vesicles. Tail reduced or absent. Small amount of body pigment. Very abnormal.
Neural tube to optic vesicle	1-1,000	13 hrs.	72	all?	31	3 days	Very retarded, reduced trunk and tail regions, very abnormal. Cycloplan-like distribution of eye pigment. Degeneration and regional differentiation common.
Non-pigmented optic cup beating heart	1-1,000	11 hrs.	35	all?	33	23 days	Retarded but some typical. Circulation abnormal or absent in more retarded cases. 6 hatched.
		27 hrs.	41	all			Slight heart beat, further development. Slight pigmentation of eyes. Larger size. None hatched.
		33 hrs.	20	all?	19		Retarded but typical. Small. None hatched.
Optic cup	1-1,000	23 hrs.	19	all	all	25 days	Typical but small. Feeble heart beat. Yolk plexus either typical, absent or showing no circulation. Died—none hatched.
		21 hrs.	33	all		10 days	Some further development. No pigment in eyes or body. Died.
		39-48 hrs.	68	all			Some differentiation during exposure. Slight pigment formation. No yolk-sac circulation. Enlarged pericardial sac. Shortened tail. None hatched.

centrations and that growth and differentiation continue, but more slowly than normal, during exposure to the strongest solutions employed. Fewer embryos died and the percentage of recovery was greater than in the case of the cleavage stages. All stages tested exhibited considerable variation in susceptibility of the individuals in a culture but this became

less marked in the oldest stages. As in the case of the cleavage stages, effects were apparent in the size of embryos, body shape, pigmentation of eyes and body, development of the tail and in the circulatory system, especially heart and yolk circulation.

A common occurrence following the exposure of younger stages to a 1-1,000 concentration was an apparent degeneration of the embryonic tissues to form a dark mass of structureless material on the surface of the yolk. At first these cases were discarded but later, on the chance of possible recovery, they were transferred to fresh water. In one case very little change was noted until 2 to 3 days following transfer when observation showed aggregated eye-pigment masses and a variable distribution of pigment cells throughout the mass. In some the heart tube had formed and was beating feebly. The slight pulsation waves originated in the posterior part of the tube and proceeded anteriorly. Large vesicle-like cavities were common. These differentiations point to specific regional survival independent of normal association. No further development occurred although the cases survived many days.

Disturbances in the vascular system often account for the early death of otherwise quite typical embryos (Paff, 1939). Generally this appeared as a failure of the yolk blood vessels to form or there were evident isolated aggregates of pink or colorless blood cells on the surface of the yolk. In more retarded cases the heart failed to form, beat very feebly without any blood passing through it, was curiously bent, twisted or looped, or departed at odd angles from the body edge. In such instances it was always contained within a large cyst-like space beneath the head.

The central nervous system, especially the brain, appeared to be less affected although irregularities appeared even here. The neural tube often was bent or folded particularly in the mid-brain region. Vesicular outgrowths of the brain were common and different portions developed disproportionately, especially the cerebral hemispheres and cerebellum. As has been mentioned above, the development of the tail was retarded or even inhibited when the remainder of the embryo appeared quite typical. In extreme cases the tail was lacking and a corresponding shortening of the trunk region followed, or the tail resembled a spindle-like rudiment often bent in peculiar ways.

As in the chick (Paff), body flexures may be obliterated or accentuated producing weird results. In the light of the information that colchicine affects mitosis, such malformations and retardations as have been described above may in part at least be the result of this effect together with irregularities in differentiation. However, these effects are not uniform throughout all parts of the developing embryo, as differential susceptibility is exhibited by different organs and regions.

SUMMARY

1. The action of colchicine on the differentiation of embryonic structures of the fish embryo has been studied by exposing four different developmental stages of *Oryzias latipes*, i.e. early cleavage to optic cup stages, to various concentrations for different lengths of time.

2. The sensitivity of both the whole organism and its constituent parts decreases with age and differentiation. Abnormalities were produced whose type and degree depend upon the concentration of the alkaloid, length of exposure and the stage of development. Concentrations of 1–100,000 to 1–1,000 were employed but the latter proved to be most useful.

3. In addition to general retardative and inhibitory effects on cleavage and development, other types of abnormalities included variation in the pigmentation of the eyes and body, cyclopic-like eyes, retardation and inhibition of the development of the tail, irregularities in the heart and yolk-sac vessels, abnormal blood formation, disturbance of body flexures especially the tail, and disturbances in the development of parts of the brain. Apparently when gastrulation occurred, it was typical.

4. In cases where development had ceased entirely during exposure and slight degeneration had set in, differential recovery was seen to take place especially in the formation of pigment and heart development.

5. The alkaloid penetrates the egg chorion very quickly. In weaker concentrations the older embryos showed some adaptation during long exposures. Some of the described effects are similar to those caused by dinitrophenol and x-rays.

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